Changes in kidney transamidinase activity during development in male and female rats

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The developmental changes in the activity of kidney transamidinase in male and female rats were investigated. The activity in both sexes increased rapidly after birth, reaching adult levels at 4 days of age. After weaning, the activity in male rats remained constant, while in female rats it declined to 60% of that in males. Thus, transamidinase is in the neonatal cluster of enzyme differentiation.

The first step in the biosynthesis of creatine in vertebrates is catalyzed by transamidinase (L-arginine-glycine amidinotransferase, EC 2.1.4.1). In the rat, the kidney contains the largest amount of transamidinase activity, and the enzyme is located on the inner mitochondrial membrane.

Changes in neonatal rat kidney transamidinase activity have been reported by Hommes et al. Fresh kidney cortex homogenates were prepared in isotonic sucrose, and the enzyme was assayed in the 20,000 g supernatants. A gradual increase in activity was observed after birth, and adult level was reached by 30 days of age. Also, Karelin measured transamidinase activity in Triton X-100 treated kidney cortex homogenates beginning five days after birth. The enzyme activity increased gradually and then decreased after weaning and finally increased again to adult level. The rats were not separated according to sex in either of the above reports.

Differences in kidney transamidinase activity between male and female rats have been reported by Fitch. Adult female rats had approximately 50% of the activity of adult male rats. Krisko and Walker reported that the lowering of enzyme activity in female rats began at puberty (30 to 40 d of age) and reached 50% by 50 to 60 d of age. Van Pilsum and Ungar have shown that the lower activity in adult female rats may be the result of estrogenic secretions. In the above reports, transamidinase activity was measured in whole kidney homogenates.

When rat kidney is homogenized in isotonic sucrose, transamidinase is not solubilized from the mitochondria. However, the enzyme is solubilized when the homogenate is treated with Triton X-100. Because of the differences in methods employed in the previously published reports on developmental changes in rat kidney transamidinase activity, we decided to measure the enzyme activity in...
male and female rats from birth to adult in whole kidney homogenate and in both the 15 000 g supernatant and particulate fractions of the homogenate. Distilled water was employed as the homogenizing medium since approximately 50% of the enzyme activity is solubilized when fresh kidney is homogenized in water and centrifuged. Whole kidney was employed because of the difficulty in separating the cortex from the medulla in neonatal rats.

Materials and Methods

Male and female rats were purchased from Harlan Sprague Dawley of Haslett, MI. The rats were fed Purina rat chow and had access to water ad libitum, and the lights were turned on at 8 a.m. and off at 8 p.m. The rats were bred and the offspring were reared. The animals were sacrificed by decapitation between 9 a.m. and 11 a.m., and the kidneys were removed and chilled on ice. One female rat was sacrificed on day 20 of gestation and the pups were removed. The fetal and newborn pups were separated according to sex, and the kidneys from several animals were pooled.

After the day of birth, fresh kidneys from individual rats were pooled prior to homogenization in 19 vol. of distilled water at 0°C with 7 passes at maximum speed with a Potter-Elvehjem Teflon pestle. The 5% homogenates were centrifuged at 4°C for 15 min at 15 000 g. The supernatant was removed, and the pellet was resuspended in the original volume of distilled water and homogenized with three passes with a Teflon pestle.

Aliquots of the homogenate, 15 000 g supernatant, and 15 000 g resuspended pellet were diluted with 4 vol. of 0.10 M sodium potassium phosphate buffer, pH 7.4, just prior to assaying transamidinase activity as described by Van Pilsum et al. (9). One unit of enzyme activity was the formation of one micromole of ornithine per hour at 37°C. The incubations were performed in duplicate, and the duplicate assays varied by less than 5%.

Results

The results shown in Fig. 1 indicated that the transamidinase activity in whole kidney homogenates from both male and female neonatal rats reached adult level within 4 d after birth (10 g body wt.). The enzyme activity in both sexes was similar until weaning (21 d, 40 g body wt.) (Fig. 1). However, the enzyme activity in female rats decreased during puberty (75-100 g body wt.) and remained at approx. 60% that of male rats (Fig. 1).

When transamidinase activity was measured in fractionated kidney homogenate, similar results were obtained as compared to whole homogenate. In both the 15 000 g supernatant and the particulate fractions, the enzyme activity increased rapidly after birth in both sexes and remained high until weaning (Fig. 2 and 3). In the supernatant fraction, the enzyme activity from female rats decreased to approx. 70% that of male rats following puberty (Fig. 2), and in the particulate fraction the enzyme activity from female rats decreased to approx. 55% that of male rats (Fig. 3).
Fig. 1. Transamidinase activity in whole kidney homogenate. Enzyme activity is in units per g kidney wet weight. Male rats (O) and female rats (χ).

Fig. 2. Transamidinase activity in the 15 000 g supernatant of kidney homogenate. Enzyme activity is in units per g kidney wet wt. Male rats (O) and female rats (χ).
Fig. 3. Transamidinase activity in the resuspended 15,000 g pellet from kidney homogenate. Enzyme activity is in units per g wet weight. Male rats (O) and female rats (X).

The difference in transamidinase activity between male and female rats following puberty was significant when measured in the whole homogenate ($t = 5.32$, $p < 0.001$), the supernatant fraction ($t = 3.64$, $p < 0.005$), and the particulate fraction ($t = 4.19$, $p < 0.005$).

Discussion

Enzyme differentiation in rat liver occurs primarily in three clusters during perinatal development: late fetal, neonatal, and late suckling (10,11). The results described in this report indicate that rat kidney transamidinase is in the neonatal cluster, since the enzyme activity increased rapidly after birth and reached adult level within 4 d (Fig. 1). In the previous reports (4,5), transamidinase activity increased gradually after birth and did not reach adult level until after weaning. The rapid increase in enzyme activity after birth shown in Fig. 1 is similar to that observed by Walker and Walker (12) in newly hatched chick liver.

The other enzymes required for phosphocreatine biosynthesis are present in fetal rats and also increase rapidly to adult levels after birth (4,5,13). Increasing amounts of creatine must be synthesized to supply the growing muscle mass. According to Hommes et al. (4) guanidinoacetate transmethylase reached maximum activity in rat liver at birth. However, according to Karelin (5) the enzyme activity reached maximum level 3 d after birth. Creatine phosphokinase activity in rat skeletal muscle was present at birth, and it increased gradually until weaning (4).
The low level of transamidinase activity in rat kidney just prior to and at birth (Fig. 1) may be the result of the high concentration of creatine found in fetal blood from the 20th to the 22nd day of gestation (13). The fetal rat can synthesize creatine, and it can obtain it from the mother. Embryonic chick liver transamidinase (12) and neonatal rat kidney transamidinase (4) are repressed when creatine is administered. In adult rats the repression of transamidinase synthesis by creatine is at a pretranslational step (14). Growth hormone and thyroxine are required for maintaining the level of the enzyme in adult rat kidney (15). The mechanism of the induction of transamidinase in neonatal rats is unknown. However, Karelin (5) has reported that the early postnatal induction of guanidinoacetate transmethylase in rat liver is mediated by glucagon and 3',5'-adenosine monophosphate.

The lowering of transamidinase activity in female rats following puberty (Fig. 1) is in complete agreement with that reported previously (6-8). Guanidinoacetate transmethylase activity is the same in male and female rats (7). Growth of female Sprague Dawley rats nearly ceases after about 70 d of age while male rats continue to grow throughout life (D. R. Owens, personal communication). Transamidinase activity in units per g of kidney remains constant in male rats while kidney and body mass increase with age. Thus, the total amount of enzyme activity per animal increases with age in male rats. In adult female rats the total enzyme activity per animal remains nearly constant. The difference in activity between male and female rats is under hormonal control (7,8).

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References